

Claims

*most claims
improperly
dependent
see
claim 38
for an
example*

1 A nucleic acid molecule encoding a pesticidal fusion
polypeptide comprising (i) a toxin domain; and (ii) a
5 heterologous binding domain capable of binding non-
specifically to a cell membrane without disrupting that
membrane.

2 A nucleic acid as claimed in claim 1 wherein the toxin
10 domain is derived from a *Bacillus thuringiensis* cry toxin.

3 A nucleic acid as claimed in claim 2 wherein the
Bacillus thuringiensis cry toxin is CryIA(b) or (c).

15 4 (Amended) A nucleic acid as claimed in claim 1 wherein the
binding domain binds carbohydrate.

5 A nucleic acid as claimed in claim 4 wherein the binding
domain has galactose or galactosyl affinity.

20 6 (Amended) A nucleic acid as claimed in claim 4 wherein the
binding domain is derived from a lectin.

7 A nucleic acid as claimed in claim 6 wherein the lectin
25 is a type two ribosome inactivating protein.

8 A nucleic acid as claimed in claim 7 wherein the binding
domain is derived from the ricin toxin B chain.

30 9 (Amended) A nucleic acid as claimed in claim 2 which
comprises all or part of Seq ID No 1 (CryIA(b)) or Seq ID No 2
(CryIA(c)) or a sequence degeneratively equivalent thereto.

35 10 (Amended) A nucleic acid as claimed in claim 2 which
comprises all or part of Seq ID No 3 (RTB1), Seq ID No 4 (RTB2)
or Seq ID No 5 (RTB3) or a sequence degeneratively equivalent
thereto. *ID*

11 (Amended) A nucleic acid as claimed in claim 9 which comprises the CryIA-RTB combination shown in any one of Seq ID No 6 (CryIA(b)-RTB1); Seq ID No 7 (CryIA(b)-RTB2); Seq ID No 8 (CryIA(b)-RTB3); Seq ID No 9 (CryIA(c)-RTB1); Seq ID No 10 (CryIA(c)-RTB2); or Seq ID No 11 (CryIA(c)-RTB3) or a sequence degeneratively equivalent thereto.

12 (Amended) A nucleic acid as claimed in claim 2 which comprises a nucleotide sequence which is a homologous variant of any of Seq ID Nos 1 to 11.

13 (Amended) A method of producing the nucleic acid of claim 1, which method comprises the step of combining nucleic acid encoding a toxin with a nucleic acid encoding heterologous binding domain, wherein said binding domain is capable of binding non-specifically to a cell membrane without disrupting it.

14 (Amended) A method as claimed in claim 13 wherein the method further comprises the step of modifying the sequence of the toxin or binding domain by way of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid.

15 A method as claimed in claim 14 wherein the modification of the sequence causes an alteration in the codon usage of the sequence.

16 (Amended) A recombinant vector comprising a nucleic acid as claimed in claim 1.

17 (Amended) A vector as claimed in claim 16 wherein the nucleic acid of claim 1 is operably linked to a promoter.

18 A vector as claimed in claim 17 which is an inducible promoter which is switched on in response to an elicitor or other plant signal which is triggered in response to predation.

19 (Amended) A vector as claimed in claim 16 which is a baculovirus vector or a vector suitable for use in a plant.

20 (Amended) A method for transforming a host cell which method

includes the step of introducing a vector of claim 16 into the cell and causing or allowing recombination between the vector and the cell genome to introduce the nucleic acid into the genome.

21 (Amended) A host cell containing the nucleic acid of claim 1.

22 (Amended) A host cell transformed with the nucleic acid of claim 1.

23 (Amended) A host cell as claimed in claim 21 which is a plant cell.

24 A host cell as claimed in claim 23 wherein the plant is a monocot plant.

25 A host cell as claimed in claim 24 wherein the monocot is maize or rice.

26 (Amended) A process for producing a transgenic plant, which process comprises the steps of:

(a) transforming a host cell by introducing a recombinant vector comprising a nucleic acid as claimed in claim 1 into the cell and causing or allowing recombination between the vector and the cell genome to introduce the nucleic acid into the genome, thereby to produce a transformed plant cell; and

(b) regenerating a plant from said transformed host cell.

27 (Amended) A plant obtainable by the process of claim 26, which comprises a host cell containing a nucleic acid molecule encoding a pesticidal fusion polypeptide comprising (i) a toxin domain; and (ii) a heterologous binding domain capable of binding non-specifically to a cell membrane without disrupting said membrane, said host cell being a plant cell.

28 (Amended) A plant which is a clone, selfed or hybrid progeny, or other descendant of the plant of claim 27.

29 (Amended) A plant as claimed in claim 27 which is a monocot.

30 A plant as claimed in claim 29 wherein the monocot is maize or rice.

31 (Amended) A part or propagule of the plant of claim 27.

32 (Amended) A method of influencing or affecting the toxicity of a plant to a pest, which method includes the step of causing or allowing expression from a nucleic acid of claim 1 in the plant.

33 (Amended) A pesticidal fusion polypeptide encoded by the nucleic acid of claim 1.

34 (Amended) A method for producing the polypeptide of claim 33 which method comprises the step of causing expression from a nucleic acid molecule, encoding a pesticidal fusion polypeptide comprising (i) a toxin domain and (ii) a heterologous binding domain capable of binding non-specifically to a cell membrane without disrupting said membrane, in a suitable host cell.

35 A composition comprising the polypeptide of claim 33 plus at least one additional component.

36 A commodity which has been treated with the composition of claim 35 such that it has a reduced susceptibility to attack by a pest.

37 (Amended) A method for controlling pests comprising treating said pests with the polypeptide of claim 33.

38 A method of assessing the toxicity of a polypeptide to a pest species comprising:

(i) introducing a nucleic acid encoding said polypeptide into a host cell from that species,

(ii) causing or allowing the nucleic acid to be expressed in a host cell from that species,

(iii) observing the viability of the cell and correlating the results of the observation with the toxicity of the polypeptide, wherein the viability is determined by assessing esterase activity or membrane integrity.

39 (Amended) A method as claimed in claim 37 wherein the pest is a species of insect.

40 A method as claimed in claim 39 wherein the species is selected from *Lepidoptera*, *Coleoptera*, *Culicidae*, *Simuliidae*, *Hymenoptera*, *Homoptera*, *Orthoptera* and *Diptera*.

Claim 41 (Amended) An oligonucleotide selected from the group consisting of:

LF1=5' CAACAACAAAGGAATTCATGCTGATG 3' (SEQ ID NO: 12),

LB1=5' GGACACACACACTGCAAGCTTGTAATC 3' (SEQ ID NO: 13),

LB2=5' CGGATCCGAAAGCTTCACATCTAACAC 3' (SEQ ID NO: 14), or

LB3=5' GCTTGCAAGCTTAGACCATATAGCCC 3' (SEQ ID NO: 15).

42 A nucleic acid as claimed in claim 10 which comprises the CryIA-RTB combination shown in any one of Seq ID No 6 (CryIA(b)-RTB1); Seq ID No 7 (CryIA(b)-RTB2); Seq ID No 8 (CryIA(b)-RTB3); Seq ID No 9 (CryIA(c)-RTB1); Seq ID No 10 (CryIA(c)-RTB2); or Seq ID No 11 (CryIA(c)-RTB3) or a sequence degeneratively equivalent thereto.

43 A host cell containing the vector of claim 16.

44 A host cell transformed with the vector of claim 16.

45 A host cell as claimed in claim 22 which is a plant cell.

46 A host cell as claimed in claim 45 wherein the plant is a monocot plant.

A host cell as claimed in claim 46 wherein the monocot is maize or rice.

48 A method as claimed in claim 38 wherein the pest is a species of insect.

49 A method as claimed in claim 48 wherein the species is selected from *Lepidoptera*, *Coleoptera*, *Culicidae*, *Simuliidae*, *Hymenoptera*, *Homoptera*, *Orthoptera* and *Diptera*.